

# Hemolymph and tissue-bound peptidase-resistant analogs of the insect allatostatins<sup>†</sup>

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## Abstract

While neuropeptides of the allatostatin family inhibit *in vitro* production of juvenile hormone, which modulates aspects of development and reproduction in the cockroach, *Diploptera punctata*, they are susceptible to inactivation by peptidases in the hemolymph, gut, and bound to internal tissues. Patterns of peptidase cleavage were investigated in two allatostatin analogs in which sterically bulky components were incorporated into the active core region to block peptidase attack. The results were used to design and synthesize the first pseudopeptide analog of an insect neuropeptide resistant to degradation by both hemolymph and tissue-bound peptidases. This pseudotetrapeptide allatostatin mimetic analog represents a valuable tool to neuroendocrinologists studying mechanisms by which the natural peptides operate and the physiological consequences of challenging an insect with an allatostatin that is not readily degraded via peptidase enzymes. Disruption of critical physiological processes modulated by neuropeptides such as the allatostatins via peptidase-resistant mimetic analogs could form the basis for novel pest insect management strategies in the future. © 1999 by Elsevier Science Inc.

Cockroach; JH synthesis; Development; Beta turn; Insect control

THE allatostatin family of peptides inhibit the *in vitro* biosynthesis of juvenile hormone (JH) by the corpora allata of the cockroach *Diploptera punctata*. A reduction in endogenous levels of JH is critical to development of the adult stage from the nymph in the cockroach whereas oocyte growth and maturation in adult females show a dependency on the presence of JH [1]. Despite their ability to modulate *in vitro* production of JH, the allatostatins hold little promise as insect control agents because of susceptibility to inactivation by peptidases in the hemolymph and gut and/or bound to tissues within the insect [1]. Therefore, the utility of the allatostatin peptides for insect control lies in the information encoded in the primary and secondary structures that provide clues as to how they interact with their target sites and with the surfaces of degradative enzymes.

Earlier structure-activity studies have shown that the C-terminal pentapeptide Tyr/Phe-Xaa-Phe-Gly-Leu/Ile-NH<sub>2</sub>, shared by all members of the *Diploptera* allatostatins, represents the 'active core' region or minimum sequence capable of eliciting inhibition of JH production *in vitro* [7,15,16]. The side chains of active core residues Phe, Leu and Tyr proved to be the most important for activity [7]. Recent studies have elucidated the primary catabolic cleavage sites of the allatostatins following incubation with hemolymph enzymes and with membrane peptidases in crude membrane preparations. Hemolymph enzymes primarily cleave the peptides in the N-terminal region outside of the pentapeptide core region and therefore, do not inactivate the allatostatins [5]. However, membrane preparations of brain, gut and corpora allata cleave allatostatins at the C-terminus

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between residues Gly-Leu, with secondary cleavage occurring between the residue block Tyr-Xaa [6]. Both of these cleavages disrupt the active core sequence and lead to completely inactive fragments.

In this study, we investigated the patterns of peptidase degradation in two allatostatin analogs in which components with a greater degree of steric bulk were incorporated within the active core region to block the approach of tissue-bound peptidase enzymes to susceptible peptide bonds within the region. The resulting data was used to design and synthesize a pseudotetrapeptide allatostatin analog with resistance to degradation by both tissue-bound and hemolymph peptidases.

## 1. Method

### 1.1. Allatostatin analog synthesis

The allatostatin analogs 396-1 (Ala-Arg-Pro-Tyr-Asn-Aic-Gly-Leu-NH<sub>2</sub>, Aic = 2-amino-indane-2-carboxyl-) and 397-2 (Ala-Arg-Pro-Tyr-Asn-Phe-Cpa-Leu-NH<sub>2</sub>, Cpa = cyclopropylAla) were synthesized as previously described [13]. The pseudopeptide allatostatin analog AST(b) $\phi$ 2 (Hca-Asn-Phe-Cpa-Leu-NH<sub>2</sub>, Hca = Hydrocinnamyl- and Cpa = cyclopropylAla-) was synthesized utilizing Fmoc protection chemistry on MBHA resin (0.97 meq/gm substitution; Advanced Chemtech, Louisville, KY). Coupling reagents used for the majority of the amino acid condensations were 1 eq. of 1,3-diisopropyl carbodiimide/1-hydroxy-7-azabenzotriazole (HOAt) mixture in dimethylsulfoxide for 2 h according to previously described procedures [11]. However, for coupling of the Cpa residue and the residue immediately following Cpa to the peptide-resin complex the reagent used was 1 eq. [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (HATU) (PerSeptive Biosystems, Marlborough, MA) with 2 eq. *N,N*-diisopropylethylamine in dimethylsulfoxide for 4 h. Removal of the N-terminal Fmoc group from the Cpa was accomplished with 20% piperidine in dichloromethane for 1 h rather than the 30 min used for the other residues. The peptide was cleaved from the resin with HF according to previously described conditions [10]. The crude product was purified on a Waters C18 Sep Pak cartridge followed by a Delta Pak C18 reverse phase column on a Waters model 510 HPLC controlled with a Millennium 2010 chromatography manager system (Waters, Milford, MA) with detection at 214 nm at ambient temperature. Solvent A = 0.1% aqueous trifluoroacetic acid (TFA); solvent B = 80% aqueous acetonitrile containing 0.1% TFA. Initial solvent consisting of 20% B was followed by Waters linear program 6–100% B over 40 min; flow rate 2 mL/min. Retention time (Hca-Asn-Phe-Cpa-Leu-NH<sub>2</sub>): 15.0 min. The pure pseudopeptide analog was analyzed and quantified by amino acid analysis under previously described conditions, revealing the following analysis: F(1.0), L(1.0) and N(O.9). A fast atom bombardment (FAB) mass spectrum was ob-

tained by adding 10  $\mu$ g of pseudopeptide sample to glycerol (1.5  $\mu$ L) on a copper probe, followed by bombardment with 8 kV Xe atoms on a Kratos MS-50 mass spectrometer (Kratos, Manchester, UK). The structural identity and a measure of the purity of the pseudopeptide was confirmed by the presence of the following molecular ion (MH<sup>+</sup>): Hca-Asn-Phe-Cpa-Leu-NH<sub>2</sub>, 607.4 (Calc. MH<sup>+</sup>: 607.32).

### 1.2. Animals

*D. punctata* females mate almost immediately following emergence. Therefore, newly emerged, mated females were transferred each day from the stock culture to an incubator and kept at 27  $\pm$  1°C. The relative humidity was approximately 50% with a 12-hour light:dark cycle. Insects were reared on Purina Lab Chow and water. Mating was confirmed by the presence of a spermatophore. Basal oocyte length was also measured (Day 5 = 1.44–1.68 mm). Only Day 5 mated females were utilized for the degradation studies. Day 7 mated females (only with oviposited basal oocytes) were the source of corpora allata for analysis of JH biosynthesis.

### 1.3. Solid phase extraction

**1.3.1. Preparation.** Pasteur pipettes were used to make C<sub>18</sub> reversed-phase columns. A glass bead was placed in the bottom of each Pasteur pipette and approximately 200 mg 125 Å C<sub>18</sub> bulk packing material was added. Glass wool was placed over the top of the packing material to hold it in place.

**1.3.2. Procedure.** Each column was washed with 1.5 mL of 0.1% BSA in 0.1% aqueous TFA, followed by 1.5 mL of 40% acetonitrile in 0.1% TFA and finally, 1.5 mL of 0.1% aqueous TFA. Samples were diluted in 0.2 mL 0.1% aqueous TFA and applied to column. Eluant was reapplied to the column 2 $\times$ . The column was washed with 1.5 mL 0.1% aqueous TFA followed by 17% acetonitrile in 0.1% aqueous TFA. The allatostatins and their metabolites were eluted with 0.5 mL 40% acetonitrile in 0.1% aqueous TFA into 12 mm  $\times$  75 mm culture tubes and were dried with a Speed-Vac.

### 1.4. Protein assay

Protein content of membrane preparations was determined by the method of Bradford [2] using BSA as standard. The average of three separate determinations was used.

### 1.5. Allatostatin degradation assay

**1.5.1. Tissue Collection.** Day 5 mated female *D. punctata* were anaesthetized on ice before dissection. Following dissection, tissues were stored in saline (0.9% NaCl pH 7.0–7.2 or cockroach saline; NaCl 150  $\mu$ M, KCl 12  $\mu$ M, CaCl<sub>2</sub>  $\cdot$  6 H<sub>2</sub>O 10  $\mu$ M, MgCl<sub>2</sub>  $\cdot$  6 H<sub>2</sub>O 3  $\mu$ M, glucose 40  $\mu$ M, HEPES 10  $\mu$ M at pH 7.2–7.4) on ice. Tissues were cleaned of fat body and trachea and guts were cleaned.

Midgut peritrophic membrane was removed, midguts cut open, the guts gently pulled through forceps and then washed 3×. Tissues were stored at  $-70^{\circ}\text{C}$  until sufficient material was collected.

**1.5.2. Membrane preparation.** Tissues were pooled and homogenized in saline on ice in microcentrifuge tubes (2.5 mL) for 2 min with an Omni hand held homogenizer. Homogenates were centrifuged at 1000 g for ten min at  $4^{\circ}\text{C}$  to remove cellular debris. The pellet was discarded. Supernatant was subsequently centrifuged at 30,000 g for 30 min. Pellet (crude membrane preparation) was washed three times in homogenization buffer and resuspended in saline using the Omni homogenizer.

**1.5.3. Hemolymph preparation.** Hemolymph was collected following the method of King and Tobe [8]. Hemolymph was diluted 100× with saline.

**Assay:** Crude membrane preparation or hemolymph was aliquoted into 1.5 mL microcentrifuge tubes for in vitro assay. Dip-AST5 (6  $\mu\text{M}$ ) or analog was added to each assay tube from a 1 mM stock solution. Total incubation volume was 500  $\mu\text{L}$ . Tubes were placed on a shaker at room temp for the duration of the incubation time. Incubations were terminated by the addition of 200  $\mu\text{L}$  30% aqueous TFA. Samples were centrifuged at 10,000 g and the supernatant was applied to a  $\text{C}_{18}$  solid phase extraction column. None of the potential, synthetic Dip-AST5 metabolites are lost (unpublished data). The 17–40% acetonitrile fraction was concentrated with a Speed-Vac apparatus and held at  $-70^{\circ}\text{C}$  until HPLC analysis. Samples were diluted in 0.5 mL 0.1% aqueous TFA, filtered through 0.2  $\mu\text{M}$  micro-spin filters and injected onto the HPLC column. Separation of allatostatins and their catabolites, or analogs and their catabolites, was performed using reversed-phase HPLC (see HPLC methods). When available, retention times of synthetic metabolites were used as a reference to identify endogenous metabolites. Dip-AST5 fragments were synthesized by the Institute for Biosciences and Technology, Dept. of Entomology, Texas A & M University. Dip-ASTs were synthesized by the Core Facility of Insect Biotech Canada (Department of Biochemistry, Queen's University) or obtained from Sigma. The identity of fragments resulting from peptidase degradation was also confirmed by FAB mass spectrometry by adding samples in a glycerol medium (1.5  $\mu\text{L}$ ) onto a copper probe, followed by bombardment with 8 kV Xe atoms on a Kratos MS-50 mass spectrometer (Kratos, Manchester, UK).

## 1.6. HPLC

Degradation experiments were analyzed by RP-HPLC using a  $220 \times 4.6$  mm Brownlee Phenyl column (5  $\mu\text{M}$ ) on a Spectra-Physics chromatography system with a Spectra-Physics Chromjet integrator and a Spectra-Physics 8490 detector. Following a 5 min wash with 15% acetonitrile in 0.1% aqueous TFA, a linear gradient of acetonitrile (15–

37.5% in 50 min; 37.5–65% in 5 min) at a flow rate of 0.5 mL/min was used to elute peptides and analogs. Fractions (0.5 mL) were collected each minute during the gradient run.

## 1.7. Radiochemical Assay

Rates of JH release were determined by the in vitro radiochemical method of Feyereisen and Tobe [4] and as modified by Tobe and Clarke [18]. The incorporation of  $\text{L}[^{14}\text{C}\text{-S-methyl}]\text{-methionine}$  (50  $\mu\text{M}$ , specific radioactivity 1.48–2.03 GBq/mmol from New England Nuclear or Amersham) into JH III at its penultimate step of biosynthesis by CA incubated in 50  $\mu\text{L}$  TC 199 (GIBCO, 1.3 mM  $\text{Ca}^{2+}$ , 2% Ficoll, methionine-free) was used to quantify JH release. Animals were anaesthetized on ice prior to dissection. Corpora allata were dissected directly into non-radioactive medium (only animals with oocyte length within the correct range were used for assay). Samples were dried with nitrogen and resuspended in 10  $\mu\text{L}$  1N HCl. Radioactive medium was added and neutralized with 10  $\mu\text{L}$  NaOH. Individual CA were transferred from non-radioactive medium to radioactive medium and were incubated for 3 h. The amount of inhibition was expressed as the percent reduction from the untreated rate:  $[1 - (\text{treated rate} / \text{untreated rate})] \times 100\%$ . Each value represents replicate incubations of a minimum of 8 tests.

## 1.8. Controls

All experiments were run with appropriate controls (Dip-AST5 incubated for 120 min with no membrane preparations added). If the identical amount of AST was incubated under the same conditions with saline alone, the size of the HPLC-detected peak (U.V. 214 nm) remained constant over a 120 min period. The addition of 200  $\mu\text{L}$  of 30% aqueous TFA completely inactivated the enzymes in the membrane preparations and in intact CA.

## 2. Result

In analog 396-1 (Ala-Arg-Pro-Tyr-Asn-**Aic**-Gly-Leu- $\text{NH}_2$ ), the Phe residue was replaced with an indane ring system (abbreviated **Aic** = 2-Amino-indane-2-carboxyl-; see Fig. 1) which preserved the presence of the side chain phenyl ring, a structural feature critical for biological activity [7]. In a second analog (397-2: Ala-Arg-Pro-Tyr-Asn-Phe-**Cpa**-Leu- $\text{NH}_2$ ), the Gly residue was replaced with a cyclopropyl ring system (abbreviated **Cpa** = cyclopropylAla-, see Fig. 1). These two analogs were part of a series containing restricted conformation components, including one with a synthetic turn mimic system, at different residue positions in the C-terminal region used in a previous study of the preferred solution conformations of allatostatin analogs. Molecular dynamics analyses, incorporating distance and angle constraints obtained from NMR spectroscopy, were conducted separately for each of the analogs of the series.

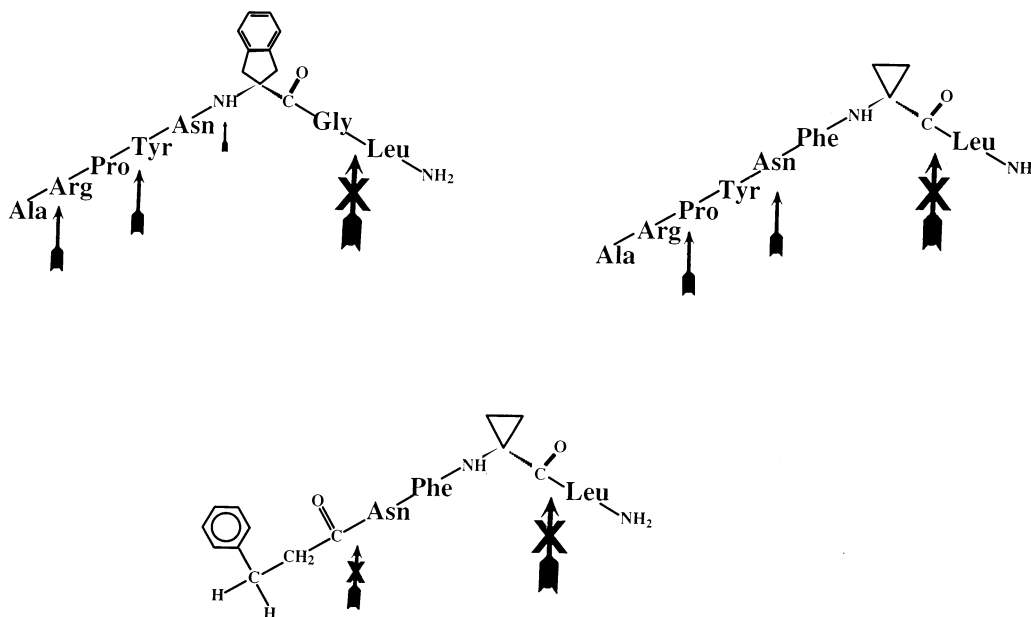


Fig. 1. Structures of allatostatin analogs containing sterically hindered, restricted conformation components: a) indane ring analog 396-1, top left; b) cyclopropyl ring analog 397-2, top right; and c) cyclopropyl ring analog with a hydrocinnamic acid 'cap' replacement for Phe [AST(b) $\phi$ 2], bottom center. Upward arrows denote cleavage sites of tissue-bound peptidases in the cockroach. The large upward arrows with a cross over them, indicate a peptidase cleavage site that is blocked by the presence of the sterically hindered components and/or presence of the hydrocinnamic acid cap.

All demonstrated a preference for a very similar low energy turn over the four C-terminal residues. In Fig. 2, a superposition of the backbone  $\alpha$ -carbons of the C-terminal pentapeptide core regions of the two analogs used in this study demonstrates the similarity of their turn conformations [14].

Both allatostatin analogs 396-1 and 397-2 retained significant bioactivity (Table 2); specifically, 396-1 has an  $IC_{50} = 2.12$  nM which compares favorably to Dip-AST5 which has an  $IC_{50} = 1.5$  nM. Patterns of peptidase hydrolysis by hemolymph enzymes for analogs 396-1 and 397-2 indicate that the analogs were targeted at peptide bonds outside of the active core region. The analogs were cleaved near the N-terminus, initially at Arg-Pro, followed by cleavage at Pro-Tyr. These sites of cleavage are directly comparable to the sites of cleavage in the natural peptide Dip-AST5 by soluble hemolymph enzymes (Arg-Leu and Leu-Tyr) [5].

Surprisingly, these analogs showed little resistance to degradation by enzymes in crude membrane preparations. Whereas the modifications successfully prevented truncation of the C-terminal Leu-NH<sub>2</sub>, both analogs were still degraded at secondary cleavage sites near the N-terminus, similar to those observed following incubation of allatostatins with hemolymph enzymes. Membrane-bound (brain and midgut) peptidases cleaved 396-1 first at Ala-Arg, followed by cleavage at Pro-Tyr and then at Asn-Aic yielding the C-terminal tripeptide (Aic-Gly-Leu-NH<sub>2</sub>). Analog 397-2 was initially cleaved at Arg-Pro followed by cleavage at

Tyr-Asn. Interestingly, we found no evidence of aminopeptidase removal of the N-terminal Ala in analog 397-2.

Since cleavage of analogs 396-1 and 397-2 occurred near the N-terminus, we synthesized analog AST(b) $\phi$ 2 (Fig. 1), which retained the cyclopropyl ring system of 397-2 at the C-terminus and also incorporated an unnatural, non-amino acid group, hydrocinnamic acid, at the N-terminus. The hydrocinnamic acid group replaces the critical N-terminal Tyr, thereby effectively removing both cleavage sites suggested from the incubation of Dip-ASTs with hemolymph enzymes. This also effectively removed the sites targeted by membrane-bound enzymes in analog 397-2. The hydrocinnamic acid moiety lacks the phenolic OH group and N-terminal amino group of the Tyr residue. We expected that this analog would combine resistance to catabolic membrane bound enzymes in crude membrane preparations and to soluble catabolic enzymes in hemolymph while retaining significant biological activity. This analog confirmed our expectations in that it exhibited extreme resistance to degradation by enzymes in hemolymph and in crude membrane preparations of brain and midgut (Table 1). Assay of AST(b) $\phi$ 2 for inhibition of JH biosynthesis indicates that it does retain significant biological activity (Fig. 3).

### 3. Discussion

Studies on the structure-activity relationships of the allatostatins indicate that the C-terminal pentapeptide Tyr/Phe-

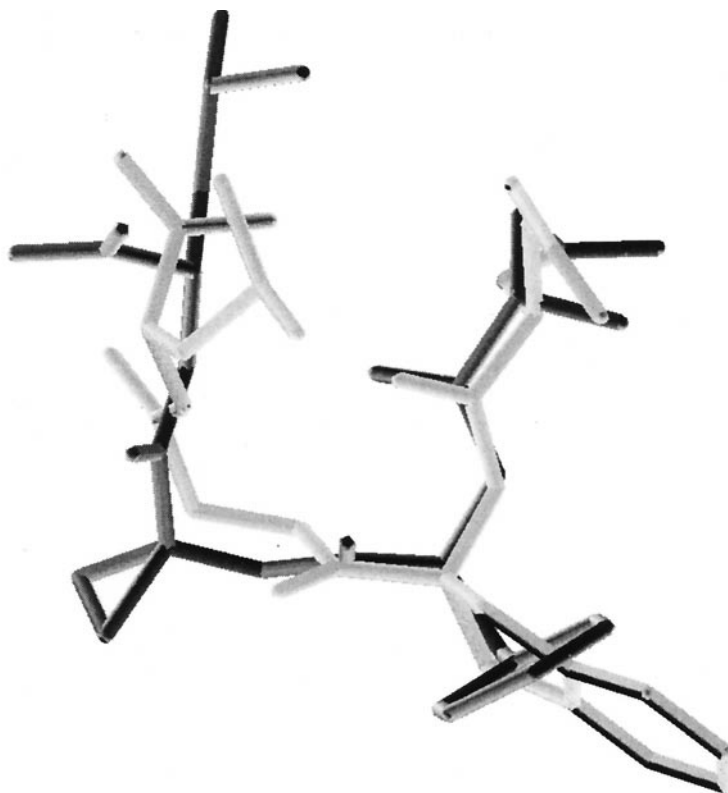


Fig. 2. A previous study of the solution conformation of a series of conformationally restricted allatostatin analogs, including one with a synthetic turn mimic component, involved molecular dynamics analyses incorporated distance and angle constraints from NMR spectroscopic experiments. The series of analogs all demonstrated a preference for a very similar turn conformation in the C-terminal region. Two of these analogs, used in this study of peptidase degradation patterns, are illustrated in this figure. Superposition of the backbone  $\alpha$ -carbons (RMS = 0.9 Å) of the C-terminal pentapeptide core regions of the minimum energy structures of indane ring analog 396-1 (light tube) and cyclopropyl ring analog 397-2 (dark tube) demonstrate the very similar nature of their turn conformations [13,14]. Sidechains have been eliminated to simplify the illustration.

Table 1

Half-life of Dip-AST5 and AST-analogs

AST/AST-analog	Haemolymph <sup>1</sup> (min)	Brain <sup>2</sup> (min)	Midgut <sup>2</sup> (min)
Dip-ASTS	153.0	18.3	67.9
396-1	ND	17.2	22.6
397-2	ND	60.9	20.8
AST(b) $\phi$ 2	1214.7	10771.5	$\infty$

Samples were extracted and separated by Phenyl RP-HPLC. Quantity of Dip-AST5 or analog remaining at the end of each incubation was determined by comparison of relative peak area to peak area of authentic. Dip-ASTS at time was determined following a 1–2 hour incubation period. Values represent the mean of at least 5 assays. ND = not determined.  $\infty$  = no degradation was observed.

<sup>1</sup> Haemolymph was diluted 100  $\times$  with saline.

<sup>2</sup> Crude tissue homogenates were prepared at 25 ng protein/ $\mu$ l saline.

Xaa-Phe-Gly-Leu/Ile-NH<sub>2</sub> represents the core sequence required for functional allatostatic activity in vitro [3,7,15,16]. In terms of the inhibition of JH biosynthesis, the amino acid side chain of Leu was the most important side chain, followed by Phe and Tyr, all of which are located in the C-terminal core sequence. Recent studies have elucidated the primary catabolic cleavage sites of allatostatins following incubation with either soluble enzymes in the hemolymph or with membrane peptidases in crude membrane preparations [5,6]. Hemolymph enzymes cleave Dip-AST5 primarily near the N-terminus at Arg-Leu, to yield the C-terminal hexapeptide. This hexapeptide is subsequently cleaved at Leu-Tyr to yield the C-terminal pentapeptide [5]. Interestingly, these cleavages do not inactivate Dip-AST5 because they do not target sites within the core sequence of the allatostatins. However, the potency of these two catabo-

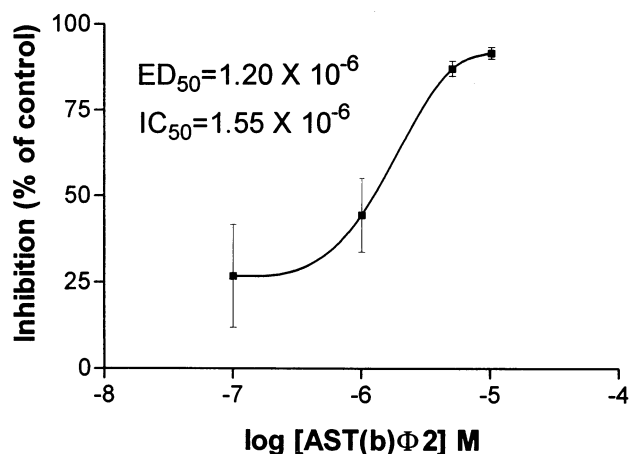


Fig. 3. Dose-response curve for inhibition of *in vitro* juvenile hormone JH biosynthesis in corpora allata of the cockroach *Diploptera punctata* by allatostatin analog AST(b)φ2. Each point for analog treatment represents the mean of 14–16 measurements  $\pm$  the standard error of the mean.

lites is significantly reduced [17]. For example, the activity of the C-terminal pentapeptide is reduced about 1000-fold [1]. Catabolic enzymes in crude membrane preparations of brain, gut and corpora allata cleave allatostatins primarily in the core C-terminal region at Gly-Leu-NH<sub>2</sub>, with a secondary cleavage site between Tyr-Xaa. Cleavage at the primary site completely inactivates the allatostatin in terms of the inhibition of JH biosynthesis. These structural and metabolic studies provided a basis for the design and synthesis of several *Diploptera punctata*-AST derived pseudopeptides, in which active-core residues adjacent to peptide bonds susceptible to degradation were selectively replaced by components with enhanced steric bulk. These regions of steric hindrance also conferred greater conformational rigidity, providing an opportunity to elucidate the active conformation, i.e., that conformation adopted by the allatostatins during successful receptor interaction. These  $\alpha,\alpha$ -disubstituted residues were expected to promote a kink in the backbone of this region of the peptide. Indeed, in a previous study [13,14], dihedral angle and NOE distance

constraints obtained from solution NMR spectra of the two analogs, along with several other constrained analogs, were incorporated into molecular dynamics calculations to ascertain solution conformation. Computer graphics illustrations of the results revealed that the whole series of analogs formed very similar turn conformations, stabilized by hydrogen bonds spanning the turn, in the C-terminal regions encompassed by the native residues Asn-Phe-Gly-Leu [14].

Analog 396-1 and 397-2 were constructed with modifications in the C-terminal 'active core' region of the allatostatins to block degradative cleavage by membrane-bound enzymes. Surprisingly, these analogs showed little resistance to cleavage by membrane-bound enzymes. Nevertheless, the two analogs did block the membrane-bound degradation that would occur at the primary cleavage site within the C-terminal active core pentapeptide region, between Gly-Leu-NH<sub>2</sub> in native peptide. The observed cleavage pattern of 396-1 by membrane-bound (brain and midgut) peptidases is consistent with aminopeptidase removal of Ala followed by two consecutive dipeptidyl aminopeptidase-like (DAP) cleavages. In analog 397-2, the two observed cleavages are also consistent with DAP-like removal of successive N-terminal dipeptides. Alternatively, the second cleavage observed with 397-2 (Tyr-Asn) may be catalyzed by a chymotrypsin-like enzyme, because it occurs on the carboxyl side of an aromatic residue.

This N-terminal cleavage pattern observed with analogs 396-1 and 397-2 provided the impetus for the design of analog AST(b)φ2, incorporating the cyclopropyl ring system from 397-2 and a hydrocinnamic acid group, a mimic of the Tyr residue, to 'cap' the N-terminus. This analog effectively removed cleavage sites suggested from incubation of Dip-ASTs with hemolymph enzymes, and those sites targeted by membrane-bound enzymes in native allatostatins and in analog 397-2. The observed results indicate that AST(b)φ2 is the first mimetic analog of an insect neuropeptide with resistance to both hemolymph and tissue-bound peptidases. This analog demonstrated eight-fold and 600-fold longer half-lives than the native neuropeptide Dip-AST5 following exposure to hemolymph and brain peptidases, respectively, and proved to be completely resistant to midgut peptidases. It represents a valuable tool to insect neuroendocrinologists studying both the mechanisms by which the allatostatins act and the physiological consequences of challenging an insect with an allatostatic signal that is not readily degraded by peptidases. Future studies will include *in vivo* biological testing of AST(b)φ2 and the synthesis and testing of an analog comprising the indane ring system of 396-1 coupled with the N-terminal hydrocinnamic acid cap.

Our results suggest that it is possible to develop analogs resistant to cleavage by catabolic enzymes. These analogs need not be as potent as the natural peptide to disrupt physiological processes [12] such as JH biosynthesis, be-

Table 2

*in vitro* inhibition of JH biosynthesis by Dip-ASTs and AST analogs

AST/AST-analog	IC <sub>50</sub> <sup>1</sup>
Dip-ASTs	3.12 × 10 <sup>-9</sup> M
396-1	2.12 × 10 <sup>-9</sup> M
397-2	1.72 × 10 <sup>-7</sup> M
AST(b)φ2	1.55 × 10 <sup>-6</sup> M

<sup>1</sup> IC<sub>50</sub>: Molar concentration of Dip-AST or AST-analog required for half-maximal inhibition of JH release in a 3 h *in vitro* radiochemical assay with single 7-day mated CA compared to groups of controls (n > 5).

cause their effects can be exerted over a considerable time, as a consequence of their resistance to peptidase degradation. Pseudopeptide analogs offer the potential to selectively manipulate and disrupt many critical physiological processes regulated by neuropeptides. Future peptidomimetics can be designed to exploit the actions of neuropeptides by increasing their potency, by rendering them resistant to catabolic enzymes, by blocking their receptors or by blocking their degradative proteases. Modern insect pest manage-

ment can now encompass the manipulation of important physiological processes by a battery of biochemical and molecular genetic techniques, including the use of pseudopeptide analogs.

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